CHAPTER 1

Literature Review

1. 1 Introduction

The j*ambu air* fruit is a tropical fruit belonging to the genus *Syzygium* of the family Myrtaceae. The genus is believed to have originated in South-East Asia although current distribution of the tree have been documented in India and throughout South-East Asia to the Pacific Islands where reportedly the Malay apple, as it is sometimes called, features in Fijian mythology (Pigram and Panggabean, 1984; Hakim and Panggabean, 1992). Relatively recently *Syzygium samarangense* has become one of the popularly grown fruits in this region.

It is fairly widely cultivated and grown throughout Malaysia, on a small scale, where the climate is very suitable for its production all year round. In Malaysia it is cultivated mainly as smallholdings ranging from 1 to 5 ha with the pale green, red (dark red), pink (light red) and green as the four major cultivars. In Indonesia the production area and production, for the year 2003, were 13,454 ha and 239,108 tons, respectively (Shu et al., 2008). Fruits are not seasonally produced, the peak months being, March to April and November to December. In 2005, in Taiwan, the area, total production and production were 7302 ha, 84,991 tons and $182 million USD, respectively (Shu et al., 2008). Pink is the leading cultivar in Taiwan. Due to the successful off-season production techniques, fruits can be harvested almost all-year-round in Taiwan. In Thailand however, the planted area was 10,240 ha and 69,608 tons fruits were generated with a production value of about $ 26.5 million USD (Shu et al., 2008).

As the agriculture sector in Malaysia is growing over recent years, there is a great deal of interest in producing quality fruits, particularly, *jambu* fruits which can fulfill the local demand as well as some foreign capital. Currently in Malaysia it is cultivated mainly as smallholdings ranging from 1 to 5 ha, with a total area estimated at 1,500 ha in 2005 (Shu et al., 2008). It has a great potential to succeed being a tasty fruit

with a rich source of minerals, antioxidants and vitamins. As consumers purchase fruits more increasingly based on their quality, it is essential to understand the physicochemical changes that takes place accompanying ripening and the postharvest period. The postharvest handling of the fruit is essential to its success in the fruit industry. The scientific literature does include a few studies on the physicochemical changes in *jambu air* fruits, however they are few and far between and neither adequate nor conclusive. The main objective of this research is to investigate, understand and further improve the potential of *jambu air* fruits which will benefit both the local farmers and the country's economy.

1.2 The Botany of *Syzygium samarangense*

The tree is commonly cultivated throughout the tropical lowlands in South East Asia where it is believed to originate from. The genus *Syzygium* consists of about 1100 tropical species. The nomenclature of the *jambu air* fruits is as follows (Morton, 1987a):

Kingdom: Plantae

Sub Kingdom: Tracheobionta (Vascular plants)

Super Division: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Order: Myrtales

Family: Myrtaceae

Genus: *Syzygium*

Species: *Syzygium samarangense* (Blume) Merr. & Perry

Common names of *S. Samarangense* include wax *jambu*, java apple (English); *jambu semarang, jambu klampok* (Indonesian); *jambu air mawar* (Malay); *makopa*

(Filipino); *chomphu-kaemmaem, chomphu-khieo* (Thai); *roi* (Vietnamese); *bellfruit* (In Taiwan) (Verheij and Coronel, 1992).

The trees of *S. samarangense* are cultivated in home gardens, often planted along driveways and paths as well as cultivated in small holdings (Fig. 1.1). The trees grow well in fairly moist tropical lowland areas up to 1200 m in elevation. They also grow best in areas with a fairly long dry season. However this does not mean that the tree is drought-resistant. In fact it requires a reliable supply of water and is often planted along small rivers, streams or ponds (Pigram and Panggabean, 1984; Hakim and Panggabean, 1992).

Figure 1.1 The *jambu air* trees taken at the study site.

The trees are around 5 to 15 meters tall with short and crooked trunks measuring 25-50 cm in diameter. The trees are often branched near the base and have a wide, irregular canopy. The leaves are opposite, elliptic to elliptic-oblong, 10-25 cm x 5-12 cm in size, curvaceous with a thin margin, pellucid and often dotted. The leaves are also

quite strongly aromatic when bruised and have thick petioles between 3-5 mm long. The inflorescences are terminal and in axils of fallen leaves with 3-30 flowers. The flowers are invariably 3-4 cm in diameter with a calyx-tube about 1.5 cm long (Fig. 1.2). They are ventricose at the apex with lobes 3-5 mm long with 4 petals. Their shape are orbicular to spathulate, 10-15 mm long and yellow to white in color with numerous stamens and a style numerous, up to 3 cm long.

Figure 1.2 Photograph of the *Syzygium samarangense* flowers.

The fruits are a berry, broadly pyriform, crowned by the fleshy calyx with incurved lobes with dimensions measuring 3.5-5.5 cm x 4.5-5.5 cm (Fig. 1.3). They are glossy, wax-like with a thin skin and light red to white in color with a white spongy flesh which is juicy, crisp, and aromatic with a subtle sweet taste resembling a common apple. The fruits need to be picked by hand and handled with care and are consumed or preserved within a few days from harvest. Seeds number 0-2, mostly suppressed and globose, up to 8 mm in diameter.

Figure 1.3 Photograph of mature fruits of *Syzygium samarangense.*

The trees have definite flowering seasons, often two, sometimes three in a year, but the timing varies from year to year. *Jambu air* commonly flowers early or late in the dry season and the fruit ripens 30-40 days after anthesis. In Indonesia and Malaysia the *jambu air* fruits are served in fruit salads (locally known as *'rojak'*) and are also preserved by pickling ('asinan'). Various parts of the tree are used in traditional medicine, and some have in fact been shown to possess antibiotic activity (Pigram and Panggabean, 1984; Hakim and Panggabean, 1992). In addition to this the wood is reddish, hard and grows to dimensions large enough for construction purposes in some countries.

Superior varieties of the fruits are known for their excellent quality. Almost all of the fruit is edible. The chemical composition of the fruits per 100 g edible portion is approximately 90% water with 0.3 g protein, 3.9 g carbohydrates, 1 g fiber and no fats. It has been reported to contain vitamin A (253 IU), vitamin C (0.1 g) and traces of vitamins B1 and B2. The energy content is approximately 80 kJ/100 g (Pigram and Panggabean, 1984; Hakim and Panggabean, 1992).

1.3 Ecology and Distribution

The trees of *Syzygium samarangense* are cultivated at home in fairly moist tropical lowlands up to 1,200 m elevation. *Jambu air* trees grow best in areas with a fairly long dry season. The trees of *jambu air* prefer heavy soils and easy access to water instead of having to search for water in light profound soils. The species require a reliable water supply and are frequently planted along streams or ponds (Verheij and Coronel, 1992).

The species presumably originates from and is widely distributed in South-East Asia. Present distribution ranges from India through South-East Asia to the Pacific Islands. The *jambu air* tree is native from Malaysia to the Andaman and Nicobar Islands where there are wild trees in the coastal forests. It has been introduced in many Asian countries. It is common in Thailand, Myanmar, Laos, Taiwan, Cambodia and Vietnam, often cultivated in India (Morton, 1987b).

1.4 Fruit Growth, Development and Harvest

Fruit growth and development involves changes in its morphology, anatomy and physiology (Chahidi et al., 2008) whilst fruit ripening is associated with dramatic changes in rind texture, color, juice composition, increase in softness due to changes in the cell walls, the metabolism of organic acids and the development of compounds involved in flavor and taste (Davies et al., 1997; Javanmardi and Kubota, 2006). The number of days from bloom to the time for harvest is a useful guide to determine the harvest time where the shape, ground color, size or sheen on the fruit are usually as indicators of maturity.

There are three common types of growth patterns among different fruits including a single, double, or triple sigmoidal curve for growth (Coombe, 1976; Trimble *et al.*, 2006) . Many fruits, such as apple (Dennis, 1986a) and banana (Israeli and Lahav, 1986), are reported to have a single sigmoidal growth curve in which there

is an initial phase of slow growth, followed by a rapid growth period, and finally a period of declining growth rate when ripening is often initiated. Other fruits including stone fruits, figs, and grapes show a double sigmoid curve in which there are two rapid growth phases (respectively named as periods I and III) interrupted by one short period of growth, namely period II. The growth pattern of the kiwifruit (*actinidia chinensis*) can be described as triple sigmoidal (Bollard, 1970).

According to Shu (Shu *et al.*, 1998), the growth of *jambu* fruits exhibits a sigmoidal growth curve. There are definite flowering seasons, often two, sometimes three in a year, in spring, summer and fall. The biggest crops are produced in the spring and fall flowering seasons. The tree flowers in May and June and the fruits ripen in August and September and the second crop is often in November and December. The flowers appear to be self-compatible and the fruit ripens around 30-40 days after anthesis (Morton, 1987c).

1.5 Postharvest Physicochemical Changes in Fruits during Ripening

Fleshy fruits have been classified into two categories: climacteric and non-climacteric (Wills *et al.*, 1984). Non-climacteric fruit will not ripen after harvest whilst climacteric fruits will ripen and get softer and sweeter after harvest. The biochemical process involved is those climacterics fruits give off large amounts of ethylene gas (a natural plant hormone) whereas non-climacteric fruits give little or no ethylene gas. Ethylene, considered as being the ripening hormone, controls ripening by coordinating the timely activation of many genes. Considerable progress has been made in the characterization of the ethylene biosynthetic pathway. Besides ethylene, other hormones and environmental factors affect the ripening process as well (Vendrell *et al.*, 2001).

Climacteric fruits can be harvested at any time between the mature and ripe stages. If the fruits are harvested as soon as they are mature, the ripening period can be

used to transport and market the fruit, and the 'shelf-life' of climacteric fruit can be extended for weeks or months, facilitating long distance trade. Climacteric fruits usually undergo dramatic changes during "ripening" and these changes have often been associated with a large increase in respiration and ethylene production (Defilippi *et al.*, 2009; Obando-Ulloa *et al.*, 2009). Avocado, mango and banana are classified as climacteric fruits whereas the pineapple, strawberry, citrus, rambutan are classified as a non-climacteric fruit. The *wax jambu* fruit (*Syzygium samarangense*) is a non-climacteric tropical fruit according to Morton and Moneruzzaman (Morton, 1987b; Moneruzzaman *et al.*, 2011).

1.5.1 Postharvest Changes in Non-Climacteric Fruits

Non-climacteric fruits do not change significantly after harvest and have mature fruits that ripen gradually, at a steady pace. These fruits should not be harvested before they are ripe because the ripening process stops as soon as they are picked. The taste, flavor and texture of an unripe fruit do not improve after harvest. Harvesting at the ripe stage implies that the fruit should be eaten soon and this means that there is little time for transport, trade and display in the market. On the other hand the harvest time may range widely, depending on the preferred quality.

Non-climacteric fruit do not exhibit the increase in respiration or the rise in ethylene production. The ripening of some non-climacteric fruit may be ethylene-independent although several studies have shown that ethylene has some effects on non-climacteric fruit such as cherries, citrus, and strawberries. Gong *et al.(Gong et al., 2002)* reported that exogenous ethylene stimulated respiration and accelerated the development of stem browning in the sweet cherry fruit. Ethylene has also been shown to be involved in the regulation of maturation and senescence in citrus fruits (Porat *et al.*, 1999a; Porat *et al.*, 1999b).

1.6 Postharvest Physicochemical Changes in Fruits

1.6.1 Color Change

Color is an important factor determining the appearance of fruits and subsequently its quality as well, as consumers are greatly influenced by the appearance of fruits. The disappearance of the green color in fruits and vegetables, as they ripen and mature, is usually the result of chlorophyll degradation and the concomitant unmasking or synthesis of other pigments such as carotenoids and anthocyanins which gives the fruits a yellow to red color (Shewfelt, 1993; Shewfelt and Prussia, 1993). In fruits like bananas there is no net synthesis of carotenoids but the loss of chlorophyll unmasks the yellow color present. Several factors and events come into play to bring about these changes, such as chlorophyllase, oxidative systems inherent in the plant cells, hormones, light and pH.

The relationship between color and degree of maturation has been widely studied and reported for fruits such as peaches, and nectarines (Mitchell, 1987; Luchsinger and Walsh, 1993), tomatoes (Choi *et al.*, 1995). Ong (Ong *et al.*, 2006) reported that the hue value increased with increasing carotenoid content in ripe jackfruit pulp.

A method for measuring the color changes have been developed and proposed for several fruits like citrus fruits (Jimenez-Cuesta *et al.*, 1981), plum (Crisosto *et al.*, 1993), pineapple (Bartolomé *et al.*, 1995). Along the same line, working with guava fruits, Mercado-Silva (Mercado-Silva *et al.*, 1998) reported the use of L*, a*, and hue values as being the best parameters for differentiating the different stages of maturation in guava fruits. These Parameters are "L" (lightness) that changes from zero (Beaudry et al.) to 100 (pure white), a* & b* values represent the levels of tonality and saturation, with +a (indicating red), -a (indicating green), +b (indicating yellow) and -b (indicating blue). They showed that in guava fruits, the L* and Hue values at harvest were higher

with increasing maturity stage; these values decreased to similar values after 7 days storage at 25°C. The a* values were different for each stage of maturity at harvest and increased after storage. Based on color a* values, the mature green and green yellow fruit were capable of reaching the same color development as yellow fruit. Later Singh (Singh *et al.*, 2006) reported that loss of chlorophyll associated in orange with the change in color from green to yellow, appeared to follow the change in the fruit color. Recently, Ribeiro et al., (Ribeiro *et al.*, 2007) carried out the determination of the coordinates L*, a*, b* characterizes pulp color in mango. They reported that positive values of a* and b*, are attributed to the carotenoids present in the pulp of mango fruit. In addition, Olmo (Olmo *et al.*, 2000) proposed the use of the formula 1000a*/ (L*b*) as a ‘‘Color Index’’ for recording the process of the loss in green color for oranges as they ripen.

1.6.2 Tissue Firmness

Firmness of tissue is another important aspect of fruit quality. It depends on the stages of fruit maturity as the fruit softens when it ripens. This occurs as selected cell wall hydrolytic enzymes such as pectinase, breaks down the cell wall matrix and allows the cells to slide more easily against each other and hence the softness in fruits on ripening (Jain *et al.*, 2003).

Textural change in ripening fruit is related with changes in cell wall composition and, particularly, in the loss of pectic substances as the production of pectic enzymes increases on ripening. It has been observed in a wide variety of temperate (King, 1990; Whitaker *et al.*, 1997) and tropical fruits (Roe and Bruemmer, 1981; Joseph and Aworh, 1991; Aina and Oladunjoye, 1993). Softening of fruits is brought about by the removal of the methyl ester groups from pectin (Rexova-Benkova and Markovic, 1976) in the cell wall by pectin methylesterase, allowing the action of polygalacturonase over the

resulting polymer producing a reduction in the intercellular adhesiveness and tissue rigidity (Huber, 1983; Sakai *et al.*, 1993). Rahman *et al. (Rahman et al., 1995)* reported that the concentration of polygalacturonase and pectin esterase in jackfruit (*Artocarpus heterophyllus* L.) was 12-fold and 40-fold higher respectively in mature fruit of the soft form compared to mature firm fruit. However, pectin modification is important in textural changes (Ahrens and Huber, 1990). An increase in the activities of the enzymes polygalacturonase and pectin methyl esterase has been shown in guava (*Psidium guajava* L.) (Jain *et al.*, 2003). Jain (Jain *et al.*, 2003) reported that among the cell wall polygalacturonase activities increased significantly from 85 units at MG (mature green) stage to 162 units/g f.wt at OR (overripe) stage. These results showed that polygalacturonase plays a major role in fruit ripening. Crisosto (Crisosto *et al.*, 1993) in an experiment on postharvest performance evaluation of plum (*Prunus salicina*) reported that fruit firmness was decreased significantly over the 9-day experiment period.

In an experiment on mangoes and avocados, Mizrach (Mizrach, 2000) showed that both avocado and mango fruit firmness diminished monotonically during storage at room temperature, from a hard fruit with a firmness value of about 90–120 N on the first day after harvest to a very soft fruit with a firmness of about 12 N at the end of 360 h of the softening process.

Singh and Reddy (Singh and Reddy, 2006) conducted an experiment in India on post-harvest physico-mechanical properties of orange peel and showed that the firmness values of orange slowly decreased during the post-harvest storage under both ambient and refrigerated conditions. Firmness decreased from 44.9 to 21.9 N and 44.9 to 28.9 N under ambient and refrigerated conditions respectively with increase in during storage from the 1st to the 10th day. They reported the decrease in firmness of the orange fruits has strong relationship with storage period and their results are in agreement with the

results reported by Olmo (Olmo *et al.*, 2000) for Lanelate and Valencia oranges, Kang (Kang *et al.*, 2002) for cucumber and Ladaniya (Ladaniya *et al.*, 2003) for sweet orange.

Ribeiro (Ribeiro *et al.*, 2007) reported that pulp firmness in mango fruit decreases with increasing ripening period as the pectin content decreases and the soluble solids content increases.

1.6.3 Weight Loss

Water is the most abundant single component of fruits and vegetables, which may account for up to 90% of the total mass. The maximum water content varies between individual fruits and vegetables, because of structural differences. Cultivation conditions that influence structural differentiation may also have a marked affect. Dry weight is commonly used to assess maturity since at physiological maturity growth is complete and full size is attained (Wills *et al.*, 1984). Mendoza (Mendoza and Wills, 1984), working with mango showed that there is a relationship between fruit weight and volume of the fruit. As it approached maturity, the mango fruit weight increased more than the volume. In such fruits, this probably occurred because its carbohydrate reserves are starch and the density of starch is very much higher than the density of the sugars (Quintana *et al.*, 1984). The density of a more mature fruit is usually higher than the increase in weight normally attributed to the increase in water content. During storage weight loss increased after each interval of storage (Crisosto *et al.*, 1993; Ladaniya *et al.*, 2003) due to water loss which will lead to higher concentration of sugars in fruits (Bhattarai and Gautam, 2006). Singh and Reddy (Singh and Reddy, 2006) reported that the percentage cumulative weight loss in oranges during storage under ambient and refrigerated conditions for 17 days, increased with increasing storage period under both ambient as well as refrigerated conditions. They also observed a higher weight loss for

fruits stored under ambient conditions and suggested it might be the result of the high rate of change in soluble sugar concentration due to the monosaccharides being used in the respiration process during storage at higher temperatures. Javanmardi and Kubota (Javanmardi and Kubota, 2006) showed that the main cause for weight loss is the high rate of transpiration in room temperature stored tomatoes. Postharvest water loss of fruits and vegetables results in fruit softening and reduced glossiness and shelf life (Smith *et al.*, 2006).

1.6.4 pH

pH is an important parameter in color changes of ripening fruits. In full-grown fruit, cells are compromised mainly of the vacuole, with the cytoplasm being reduced to a thin layer compressed between the tonoplast and cell wall (Kays, 1999). The vacuole accumulates organic acids, sugars and phenolic compounds including anthocyanin pigments. The accumulation of organic acids results in a buffered solution. Although this buffering capacity was measured by Holcroft and Kader (Holcroft and Kader, 1999) in strawberry. They reported an increase in pH over the 10-day storage period and a decrease in titratable acidity (TA) in the juice of strawberry. Since pH has a profound effect on anthocyanin stability and color expression, particularly in an aqueous solution, changes in pH could result in significant losses in color. Minor changes in pH can have a significant consequence on the colour expression of anthocyanin since the acidity of the solution affects the ratio between the various forms of the pigments (Holcroft and Kader, 1999). It is reported that in citrus fruit juice, the pH was 2.1 to 3.0 at the first sampling date and slightly increased with time to reach 3.0 to 3.5 (after 3 months storage) (Chahidi *et al.*, 2008).

Changes in color and the stability of anthocyanins have been reported over pH range 2.0–8.7 in tamarillo fruit (*Solanum betaceum* Cav.) (Hurtado *et al.*, 2009). The

color variation in the aqueous solutions of anthocyanin crude extracts was within the pH range 2.0–6.2, that is to say under and above the most common pH values in foods (Hurtado *et al.*, 2009).

Some studies have suggested that pH and mineral composition, may affect the catalytic activity of cell wall enzymes (Huber and O'Donoghue, 1993; Chun and Huber, 1998; Almeida and Huber, 1999). The acidification of the apoplast over the pH range can provide a mechanism for the regulation of the catalytic activity of cell wall enzymes (Pinheiro and Almeida, 2008). Pinheiro and Almeida (Pinheiro and Almeida, 2008) reported that pH affected pectin dissolution and pericarp softening. In a study on the physical and chemical changes during ripening of blackberry fruits it was observed that the acidity was inversely correlated to pH (Tosun *et al.*, 2008). The ripened sample which had a low acid content had a correspondingly high pH.

1.6.5 Total Soluble Solid Content (TSS)

Soluble solids include the soluble sugars sucrose, glucose and fructose as well as acids. Total soluble solids (TSS) or sugar content is considered to be an important parameter of quality attribute for many fresh fruits (Lu, 2004). TSS is also an important parameter to consider determining the time of harvesting. Increase in soluble solids could be attributed to the decomposition of the cell wall which causes release of water-soluble components (Rees *et al.*, 1981). Reaves (Reaves, 1959) reported that the increase in total soluble solids is probably due to the increase in water-soluble galacturonic acids from the degradation of pectic substances by polygalacturonase (PG). Sharaf (Sharaf and El-Saadany, 1987) indicated that the increase in soluble solids content in guava could be attributed to the conversion of starch to sugars. Increase in total soluble solids (TSS) and decrease in acidity are some indicators of sweetness of fruits such as mango (Lakshminarayana, 1980) Various researchers (Fuchs *et al.*, 1980;

Brown *et al.*, 1984; Medlicott *et al.*, 1990; Perkins-Veazie *et al.*, 1996) reported increase in total soluble solids (TSS) for mango and blackberries in during ripening. Holland (Holland *et al.*, 1999), also showed that the total soluble solids content increased during maturity of Fortune mandarins fruit, whereas the acidity decreased. Islam Sariful (Islam Sariful *et al.*, 2001) reported that the percentage TSS of banana increased during ripening. They explained that increase in TSS of fruits might be attributed due to increase in soluble sugars, soluble pectin and soluble organic acids. In sweet orange total soluble solids declined during storage up to 45 days and then increased at the interval of 75 days (Ladaniya *et al.*, 2003). Javanmardi (Javanmardi and Kubota, 2006) showed that during maturation and ripening of tomato fruits there were changes in total soluble solid such as the ratio of glucose to fructose and organic acids during storage. They also observed that there was no significant change in the amount of TSS both in room temperature and low temperature stored tomatoes. The range for TSS for both room temperature and low temperature stored tomatoes was 5.0–5.1%. Ribeiro (Ribeiro *et al.*, 2007) reported that the total soluble solids content increases in mango during ripening.

It has also been reported that that during ripening there was a slight and insignificant increase in the soluble solids content in blackberry at the green and red ripening stages. However at the ripened stage, the change in soluble solids was significantly different ($P < 0.01$) (Tosun *et al.*, 2008).

1.6.6 Titratable Acid Content (TA)

Juice acidity is an important parameter in defining quality. Total acidity which is also loosely referred to as titratable acidity is a measure of the total acid in the fruit. It is related to pH but the concepts are not identical. While pH measures acid strength or proton concentration, TA measures the amount of acids present and these are generally

weak acids such as malic acid in apple, citric acid in citrus, oxalic acid in rhubarb, tartaric acid in wine and lactic acid in sour milk, which do not contribute much protons in solutions and thus although being acidic, do not change the pH drastically.

Fruit taste is a balance between acids, sugars and volatiles present. Holland (Holland *et al.*, 1999) showed that the total soluble solids content increased during maturity of Fortune mandarins fruit, whereas the acidity decreased probably due to increasing water content and size of the fruit. He also suggested it could be due to the use of the acids as respiratory substrates. Clementine fruit that has less than 0.8% acidity is more and more considered of low quality as the sugars triumph over the acids and thus the fruit has an insipid taste and is also more prone to post harvest decay organisms. Decrease in total acidity and increase in TSS and total sugars during storage at room temperature was also observed by Kulkarni and Aradhya (Kulkarni and Aradhya, 2005). Kulkarni (Kulkarni and Aradhya, 2005) reported that the highest titrable acidity (0.56 as % citric acid) was recorded in 60 day-old pomegranate fruit arils. This was followed by a continuous, but significant decrease in titrable acidity to the lowest concentration of 0.33 (as % citric acid), which was recorded in 140 day old fruits. Ong (Ong *et al.*, 2006) working on jackfruit, observed that the titrable acidity throughout ripening process was in the range of 0.3–0.9%. Bhattarai (Bhattarai and Gautam, 2006) also reported that the acid in the fruits decreased during storage periods. However, Tosun (Tosun *et al.*, 2008) showed that titratable acidity increased during development in blackberry, but decreased in ripe fruits. The change in total titrable acids during storage was probably mainly due to the metabolic activities of living tissues during which depletion of organic acids takes place.

In addition Chahidi (Chahidi *et al.*, 2008), reported that juice titratable acidity of citrus fruit ranged between 0.8 and 1.5% (in during 3 months storage), which had the

greatest value (3.2%) at the first date of harvest, and declined with time as fruit over matures.

Acidity is also perceived in degrees of sourness and decreases as the grapes become ripe. Tartaric acid is the primary acid, but others such as malic and citric can be found as well. As the harvest date draws near, TA in the fruit drops (due to the respiration of malic acid). It is important to pick the fruits with enough TA or an adjustment will need to be made.

1.6.7 Total Sugar Content

One of the most important biochemical changes during ripening in fruits is the increase in sugar concentration and this decisive factor has also been often used in quality determination. Mono and disaccharides with a sweet flavor are commonly called sugars and are present in a significant amount in fruits and their derivatives, glucose, fructose and sucrose being the main sugars which can be found in fruits and fruit juices (Rambla *et al.*, 1997).

The sweetness and energy content of fruits depend to a large degree on its sugar to acid ratio. Therefore, an increase in the content of the simple sugars usually brings about a sweeter fruit especially if this is accompanied by a decrease in the organic acid and phenolics content to minimize acidity and astringency (Nair and Tung, 1980). In most fruit, there are high percentages of fructose, glucose and sucrose during ripening and there may be an absence of very minute amounts of free sugar during the immature stages. There is an increase in free sugar content (fructose, glucose and sucrose) of fruit with increasing ripeness (Jordan *et al.*, 2000; Ong *et al.*, 2006). Jordan (Jordan *et al.*, 2000) has shown that starch lost during ripening was accounted for by the increase in the glucose and fructose sugar pools in kiwifruit. Chan (Chan *et al.*, 1979) reported that sucrose made up less than 18% of the total sugar content in mature green *Carica*

papaya (110 days after anthesis). However, it increased rapidly to make up 80% of the sugars, 25 days later (fully ripe stage).

Sucrose is also the predominant component in coconut sugar, although variation in sugar content has also been reported between cultivars and stage of maturity of palm in florescence (Neto *et al.*, 1997).

Mizrach (Mizrach, 2000) reported that the results of measurements of the chemical changes in harvested mango fruits confirmed that the sugar contents and acidity followed expected trends with storage time: the sugar contents increased while the acidity decreased.

In an experiment carried out on the effect of post harvest treatments with some coating materials on the shelf life and quality of banana showed that an increase in total sugar content of banana was observed in all the treatments up to ripening stage 5 (fully ripened) (Islam Sariful *et al.*, 2001). They also explained that the increase in total sugar of fruits might be attributed to the conversion of starch to sugar.

Bashir (Bashir and Abu-Goukh, 2003), reported a remarkable increase in total sugars in guava was attributed to an increase in the activity of enzymes responsible for starch hydrolysis and the decline in the rate of sugar breakdown by respiration.

Adao (Adao and Gloria, 2005) also reported that in banana the green fruit had high starch and low soluble sugars levels. Starch levels decreased significantly throughout ripening whilst fructose and glucose levels increased.

Kulkarni (Kulkarni and Aradhya, 2005) working on pomegranate arils showed an increase in concentration of TSS, total sugars and reducing sugars during fruit development. The lowest TSS (13%), total sugar (12.6%) and reducing sugar (12.2%) contents were recorded in 40 day-old fruits. A significant increase in all of the above three constituents was recorded after the 80th day of fruit development and the highest

TSS (15.3%), total sugar (16.6%) and reducing sugar (15.7%) contents were recorded in 140 day-old fruits.

The changes in sugar content with fruit development in oriental melon showed that glucose and fructose were the major sugar of these fruits from 10 to 30 days after pollination. However a very small quantity of sucrose was detected. The changes in sugar content gradually increased from 10 to 30 days after pollination, and doubled 35 days after pollination. The change in sucrose content of fruits at 30 days after pollination was 0.2–0.6 mg g^{-1}. However the sucrose content at 35 days after pollination rapidly increased up to 40.7–45.1 mg g^{-1}. The sucrose content quickly increased in full ripens fruit (Shin *et al.*, 2007).

While respiration represents the glycolytic carbon flux, the rate of sugar accumulation represents gluconeogenic carbon flux. During the ripening process, carbon is simultaneously shunted in both directions. Rapid fluctuation of fructose 2,6-bisphosphate in pea and banana tissue may represent one means of fine control of the glycolytic pathway. Fructose 2,6-bisphosphate stabilizes the enzyme ATP-phosphofructokinase which favors glycolysis and inhibits fructose bisphosphatase which favors gluconeogenesis (Beaudry *et al.*, 1989).

1.6.8 Total Protein Content

Proteins are vital parts of organisms and contribute in every process within cells. Many proteins are enzymes that catalyze biochemical reactions that are essential to metabolism. About 4,000 reactions are known to be catalyzed by enzymes. A group of enzymes such as the polyphenoloxidases (Lattanzio *et al.*, 2001), that catalyzes the oxidation of polyphenolic compounds by molecular oxygen, are responsible for enzymatic browning reactions happening during harvesting, handling, processing and storage of many plants (Robb, 1984; Sheptovitsky and Brudvig, 1996). PPO and phenolics are responsible for some of the enzymatic browning in many fruits and

vegetables (Mayer and Harel, 1979). It has been reported that levels of PPO and phenolics may change during fruit development and ripening which may influence the potential damage, in loquat fruit (Vamos-Vigyazo and Nadudvari-Markus, 1982; Sanchez-Ferrer *et al.*, 1989). Ayaz (Ayaz *et al.*, 2008) reported that the total phenolic content decreased, while PPO activity increased in fruits during ripening. This reverse change between PPO activity and total phenolics in medlar fruits during ripening and over ripening can be attributed to an increase in the catalytic efficiency of the enzyme during ripening.

Other groups of enzymes are the antioxidant enzymes such as, superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and monodehydroascorbate reductase (MDHAR) that are part of the antioxidant defense system against the damaging effect of reactive oxygen species (ROS) (Mondal *et al.*, 2004). Mondal (Mondal *et al.*, 2004) reported that antioxidant enzyme activities increased in during the ripening of tomato but not until the later stages of ripening. Lysyl oxidase (LOX) activity increased in tomato fruits during ripening (Mondal *et al.*, 2004). During plant senescence, LOX activity increases, while SOD, CAT and POX activities fall, resulting in a concomitant decline in the ability to scavenge free radicals. They also reported that increases in lipid peroxidation products during later stages of fruit ripening could also be mediated through increased LOX activity.

Increases in lipid peroxides (Vincent *et al.*, 2001) have also been documented during ripening in fruits and evidence of a small redistribution of potassium between cell compartments which indicates leakage, has also been observed in tomato (Brady, 1987).

These changes are primarily duo to enzymatic reactions and thus, energy demand especially for protein synthesis increases during ripening (Brady *et al.*, 1970).

Since respiration is closely linked to the generation of energy, this increased demand will correspondingly increase respiratory activities which can be perceived as the climacteric. Therefore, it is apparent that one of the requirements for ripening is the adequate supply of respiratory substrates.

Some studies have determined the total protein content in some fruits. In pomegranate arils for example it has been shown that the highest total protein (209 mg/100 g) was observed in 20 day-old fruits followed by a rapid decrease (66.9 %) in total protein up to 80 days of fruit development (Kulkarni and Aradhya, 2005).

The amount of protein in some berries and fruits, vary significantly and the following data has been reported; Myrtus berries (0.9g protein/100g), blueberry (0.7g/100g), strawberry (0.6g/100g), raspberry (1.2g/100g), sweet cherry (1.1g/100g), grape (0.7g/100g), apple (0.3 g/100g) and orange (0.9g/100g) (Gebhardt *et al.*, 2005).

An experiment on guava seed has shown that a large proportion of the protein content (~86–90 g/100 g), corresponding to the glutelin fraction, is obtained as insoluble residue (Bernardino-Nicanor *et al.*, 2006). The remaining proteins (~14 g/100 g) are distributed into globulins (~10 g/100 g), and albumins and prolamins (E2 g/100 g each one).

1.7 Antioxidant Activity in Fruits

Antioxidants are substances that are capable of neutralizing the damaging. harmful molecules called free radicals that are chemically active atoms or molecular fragments that have a role in both plant and animal ageing process, chronic diseases as cancer, heart disease, stroke, Alzheimer's disease, and atherosclerosis. Free radicals containing oxygen, known as reactive oxygen species (ROS), are the most biologically significant free radicals. ROS are partially reduced forms of oxygen such as singlet oxygen, superoxide anions ($O_2^{\cdot -}$), hydroxyl radicals ($OH^{\cdot -}$) and hydrogen peroxide (H_2O_2) (Abassi *et al.*, 1998; Asada, 1999). Reactive oxygen species (ROS), are capable

of causing damage to DNA, have been related with carcinogenesis, coronary heart disease, and many other health problems related to advancing age (Cadenas and Davies, 2000; Marnett, 2000; Uchida, 2000).

An antioxidant protects the cell from the harmful effects of free radicals and when an antioxidant reacts with a free radical, it yields an electron, is oxidized, and becomes a weak, non-toxic free radical that is stable and unable to propagate the reaction (Robles-Sanchez *et al.*, 2007). Natural antioxidant defense system include enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and monodehydroascorbate reductase (MDHAR) and water soluble compounds such as ascorbic acid, glutathione, phenolic compounds and flavonoids and lipid soluble compounds like carotenoids and tocopherols, etc., help in scavenging of ROS (Foyer *et al.*, 1994).

There is increasing awareness among consumers of the importance of natural antioxidants, especially from fresh fruit and vegetables. Epidemiological studies have shown that frequent consumption of natural antioxidants is related with a lesser risk of cardiovascular disease and cancer (Renaud *et al.*, 1998; Temple, 2000). Natural antioxidants in fruits and vegetables are classified in three major groups: vitamins [vitamin E (tocopherol), vitamin C (Ascorbic acid) and ß-carotene], phenolics, and carotenoids. Hydrophilic antioxidants are Ascorbic acid and phenolics, while carotenoids and tocopherols are known as lipophilic antioxidants (Halliwell and Gutteridge, 1995). In addition, there are also several antioxidant enzymes, including catalase, superoxide dismutase (SOD), and glutathione peroxidase, that counteract many types of free radicals in the body. Leong (Leong and Shui, 2002) in an experiment on investigation of antioxidant capacity of fruits in the Singapore markets reported that

ciku (*Manilkara zapota*), had the highest antioxidant capacity among Singapore markets fruits.

Guava (*Psidium guajava* L.), also known locally as *jambu batu*, is rich in ascorbic acid (vitamin C), at levels far higher than most imported and local fruits. The fruit, in particular the pink flesh cultivar, has a fair amount of vitamin A (beta-carotene) and also has a high quantity of antioxidants such as phenols (Lim *et al.*, 2006). Other fruits such as banana (Mokbel and Hashinaga, 2005), pomegranate (Kulkarni and Aradhya, 2005), black caraway, carrot, cranberry (Yu *et al.*, 2005), tomato (Javanmardi and Kubota, 2006; Toor and Savage, 2006), apple (Maffei *et al.*, 2007), cocoa beans (Othman *et al.*, 2007), blood orange (Kelebek *et al.*, 2008), Chinese bayberry fruit (Zhang *et al.*, 2008), and *Phyllenthus emblica* L. fruit (Luo *et al.*, 2009), are also considered to be good sources of natural antioxidants.

As a result of the above, several quantitative studies investigating the phenolic content and antioxidant potential of edible fruits have been carried out and widely reported, since the role these factors play in health and disease chemoprevention are important for human well being. It has led to an upsurge of interest in phytochemicals as potential new sources of natural antioxidants. From the literature, previous phytochemical studies of the leaves of *S. samarangense* have shown the presence of ellagitannins (Lee *et al.*, 1992), flavanones (Liu *et al.*, 2005; Maurya and Yadav, 2005), flavonol glycosides (Ross *et al.*, 2005), proanthocyanidins (Hosseinian and Mazza, 2009), anthocyanidins (El Gharras, 2009; Molan *et al.*, 2009), triterpenoids, chalcones (Srivastava *et al.*, 1995; Resurreccion-Magno *et al.*, 2005), and volatile terpenoids (Wong and Lai, 1996).

1.7.1 Phenolic Content

The phenolic content in food, fruit, vegetable and beverages has been correlated with a reduced incidence of several diseases (Martha-Estrella *et al.*, 2008). There is

considerable epidemiological evidence indicating a relationship between fruit and vegetable rich diets and a decreased risk of certain forms of cancer. The role of polyphenolic compounds from higher plants as antioxidants, antimutagenic, antiinflammatory and antimicrobial agents is widely recognized (Lee *et al.*, 1995; Hatano *et al.*, 2002). The health impact of antioxidants in foods and the hazardous effects of synthetic preservatives have led to active research in the field of natural antioxidants.

Phenolic compounds represent the largest group of plant secondary metabolites that embrace a variety of structural classes (e.g. as precursors of lignin) and biological functions. Phenolic compounds include a great diversity of compounds derived from the aromatic amino acids phenylalanine and tyrosine and they are widely distributed in fruits, vegetable, seeds, medicinal plants and herbs The general characteristic of the compounds within this group is to have aromatic rings with variable degrees of hydroxylation (Fig. 1.4) (Shahidi and Naczk, 1995). The phenol subunits are classified in two groups which include simple phenols and polyphenols. The simple phenols contain phenolic acids or phenols with a carboxyl group underlying the specificity of their function. Polyphenols have at least two phenol rings such as flavonoids (Marinova *et al.*, 2005).

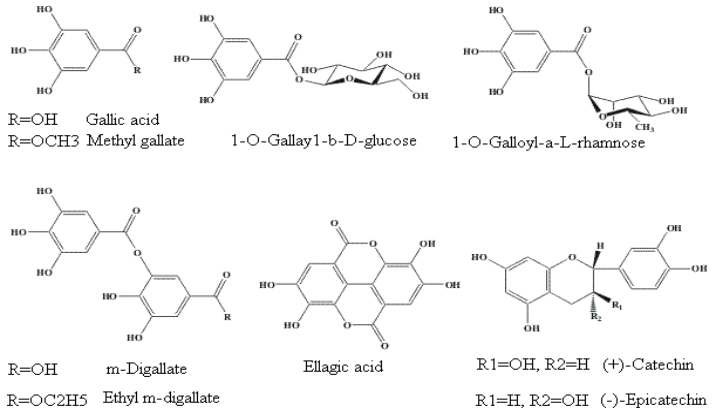


Figure 1.4 Phenolic compounds (Rice-Evans *et al.*, 1996; Tomas-Barberan *et al.*, 2000; Cuyckens and Claeys, 2004)

In plants, they are important for pigmentation. Polyphenolic anthocyanins are responsible for the blue, red, orange, purple and violet colour of plants and plant products. Phenolics are a source of natural antioxidants and are important in food and biological systems because they are preferentially oxidized, thus sparing nutrients, cells and tissues (Imeh and Khokhar, 2002). The antioxidant activity of polyphenols is higher than monophenols. Polyphenols scavenge superoxide and hydroxy radicals, inhibit lipid peroxidation, prevent oxidative damage to important biological membranes and reduce lipid peroxyl radicals (Mayo *et al.*, 2003). The beneficial properties of berry fruits on human health have been associated in part with the presence of relatively high levels of phenolic compounds (Seeram *et al.*, 2006).

Cocoa bean and its products, cocoa liquor, cocoa powder, and dark chocolate, are food sources rich in phenolic compounds. Cocoa beans have a high phenolic content of about 12–18% (dry weight) in unfermented beans (Kim and Keeney, 1984). It is reported that 60% of the total phenolics in raw cocoa beans are flavanol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer) (Dreosti, 2000). These compounds have been reported to be potential candidates to combat free radicals, which are harmful to our body and food systems (Adamson *et al.*, 1999).

In a study on Bulgarian fruits and vegetables has shown that the highest total phenolic content (TPC) is in blueberries (670.9 mg gallic acid equivalents (GAE)/100g), dogwood berries (432.0 mg GAE /100g) and sourcherry (429.5 mg GAE / 100g). The lowest TPC were observed in peaches (50.9 mg GAE / 100g). For vegetables the highest phenolic content were in green peppers (246.7 mg GAE / 100g) and red peppers (173.2 mg GAE / 100g) and lowest was in leek (stem) (27.7 mg GAE /100g) (Marinova *et al.*, 2005).

Righetto (Righetto *et al.*, 2005) in an experiment on acerola (*Malpighia emarginata* DC) reported that the total phenol contents decreased during ripening, from

3.8 mg of catechin /g for immature acerola juice to 1.4 mg of catechin/g in mature acerola juice. Ninfali (Ninfali *et al.*, 2005) working on vegetables, showed that the phenolic content matched the concentration of the two phenolic subgroups, namely, flavonoids and flavanols. They also showed that the diversification and the combination into salads of different vegetables provide an opportunity to introduce a variety of phenolics with the possibility of markedly increasing the total antioxidant capacity of the vegetable portion.

Kondo (Kondo *et al.*, 2005) also reported that the total phenolic content of guava, mango, and rose apple skin were higher than in the flesh and at the immature stage and decreased as the fruit ripened. In contrast, total phenolics of banana pulp were higher than in the skin, while those of papaya skin and flesh showed no significant differences. The composition of polyphenolics varied among fruit, and except for bananas, the skin contained a greater range of polyphenolics than the flesh.

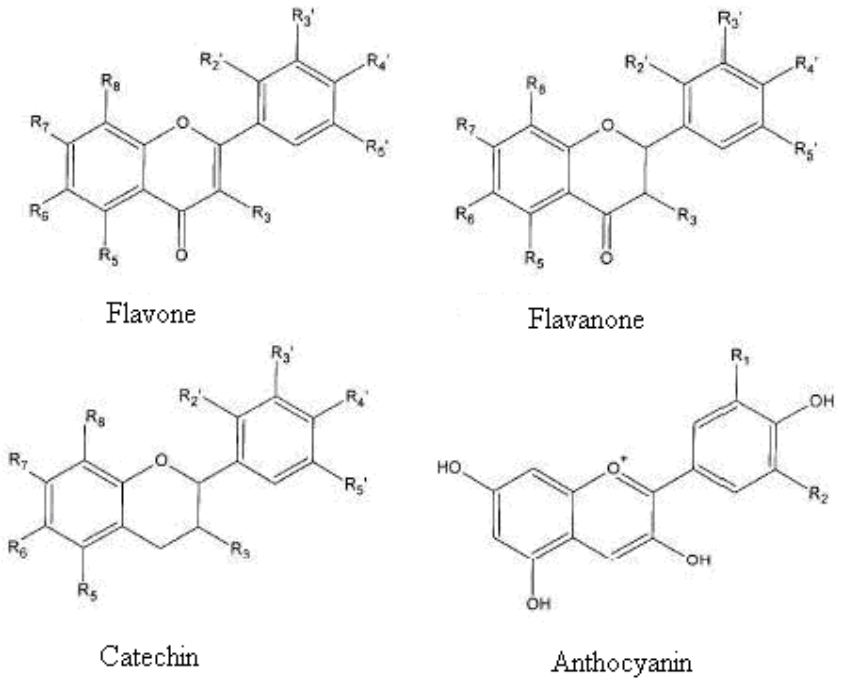
In an experiment carried out by Kulkarni (Kulkarni and Aradhya, 2005) pomegranate arils showed a rapid and significant ($P \leq 0.05$) depletion (by 54.5%) in total phenolics during the initial stage of fruit development from 20 to 40 days. At the later stages, the decrease was gradual but significant up to 140 days. The highest phenolic content (506 mg/ 100 g arils) was recorded in 20 day-old fruits. There was nearly a 73.9% reduction in total phenolics from 20 to 140 days of fruit development.

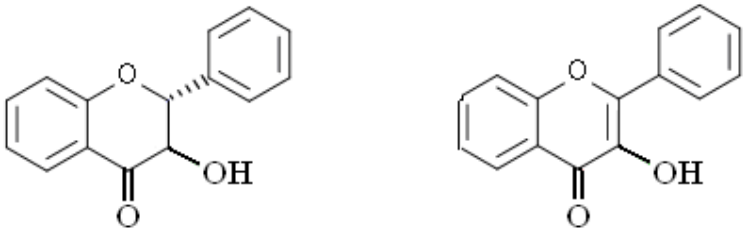
It was reported that the total phenolic content (TPC) in guava was high compared to other fruit crops (Thaipong *et al.*, 2006). The ranges of TPC (mg/100 g) in other fruits were 14–102 in nectarines, 21–111 in peaches and 42–109 in plums (Gil *et al.*, 2002), 142.9 in starfruit, 47.9 in pineapple, 56.0 in mango, 57.6 in papaya, 28.8 in litchi (Luximon-Ramma *et al.*, 2003). In a study on some Thai culinary plants it was shown that the red bird chili had the highest amount of phenolic compounds (3.48 mg

GAE / g) and the pumpkin had the lowest amount of phenolic compounds (Wangcharoen and Morasuk, 2007).

1.7.2 Flavonoid Content

Flavonoids are widely distributed in plants fulfilling many functions including producing yellow or red/blue pigmentation in flowers. They also play a role in protecting the plants from microbe and insect attacks. They are also referred to as bioflavonoids and are secondary metabolites, meaning they are organic compounds that have no direct involvement with the growth or development of plants. There are different classes of flavonoids (Le Marchand, 2002) such as: a) flavones and flavanols; b) flavanones, flavanols; c) isoflavones; d) proanthocyanidins; and e) anthocyanidins (Fig. 1.5). Over 4000 different flavonoids occurring in plants have been described (Hollman, 2001).





Favanonol Flavonol

Figure 1.5 Flavonoids include: Flavone, Flavanone, Catechin, Anthocyanin, Flavanonol and Flavonol (From: (Silva *et al.*, 2002; Farkas *et al.*, 2004))

Flavones include rutin, luteolin and apigenin, while the most abundant flavonols are quercetin and kampferol (Manach *et al.*, 2004). Onions are rich in these compounds. Blueberries also have high levels, especially in the peel, because synthesis is stimulated by exposure to light. Celery is a good source of flavones. Flavones are also present in citrus, but they are associated mainly with the fruit peel.

Isoflavones are phytoestrogens present in legumes. Soybean products are a good source of these compounds (Manach *et al.*, 2004). The three most commonly found isoflavones are genistein, glycitein and daidzein. Proanthocyanidins are oligomeric flavonoids (usually dimers or oligomers of the flavanols catechin and epicatechin). They are common in the peel and seeds of grapes (Gu *et al.*, 2004). Other sources of these compounds include apple, almond and blueberry.

Anthocyanidins are pigments giving several fruits their characteristic red or purple colors, although in some conditions they can be uncolored. Besides being pigments, anthocyanidins have great relevance due to their contribution to the antioxidant capacity of fruits and vegetables.

Fruits and vegetables are high in flavonoid content. Flavonoids impart color and taste to flowers and fruits, and it is estimated that humans consume between a few

hundred milligrams and one gram of flavonoids every day (Hollman and Katan, 1999; Pietta, 2000).

Fruit phenolic compounds include mainly flavonoids (e.g. flavonols, flavones, isoflavone, anthocyanins, flavanones, chalcones), phenolic acids, quinones, and tannins (Sakakibara *et al.*, 2002). In an experiment on dates it was reported that the total flavonoid content (TFC) varied considerably from 1.62 to 81.79 mg in terms of catechin equivalents/100 g of sample (Biglari *et al.*, 2008). Zhang (Zhang *et al.*, 2008) reported that the antioxidant capacity also increased with the increase in fruit maturity at harvest in Chinese bayberry. These changes were well correlated with the increases in the contents of total phenolics, flavonoids and anthocyanins.

In other fruits the biosynthesis of different anthocyanin types and other flavonoids may continue after harvest and during air storage even at low storage temperature in dark conditions as was found in blueberry (Kalt and McDonald, 1996), pomegranates (Holcroft and Kader, 1999) and strawberry (Kalt *et al.*, 1993; Holcroft and Kader, 1999). Increasing the CO_2 concentration around fruit inhibits the postharvest increase in anthocyanin, by affecting its biosynthesis and or its degradation or both (Holcroft and Kader, 1999). In strawberry fruit, the concentrations of other phenolics as ellagic acid, catechin, quercetin and kaempferol derivatives also increased during storage but these were not affected by CO_2 concentration in the storage atmosphere (Holcroft and Kader, 1999). Tomas-Barberan (Tomas-Barberan *et al.*, 2000) observed an increase in anthocyanin concentration in nectarine, cherry, grape and strawberry fruits during air storage whereas flavonoids and hydroxycinnamic acid derivatives remained constant except for resveratrol in grapes and ellagic acid in strawberry, which increased.

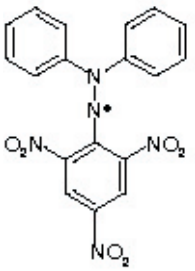
1.7.3 Antioxidant Assays

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products and foods for clinical studies including the 2,2- azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS assay) (Miller and Rice-Evans, 1997; Leong and Shui, 2002), and the 2,2- diphenyl-1-picrylhydrazyl (DPPH assay) (Brand-Williams *et al.*, 1995; Gil *et al.*, 2002), ferric reducing antioxidant power (FRAP) (Benzie and Szeto, 1999; Jimenez-Escrig *et al.*, 2001; Guo *et al.*, 2003), and the oxygen radical absorption capacity (Llorach *et al.*, 2002) (Cao *et al.*, 1993; Ou *et al.*, 2001). The ORAC assay is said to be more relevant because it utilizes a biologically relevant radical source (Penza *et al.*, 2007). These techniques have shown different results among crop species and across laboratories.

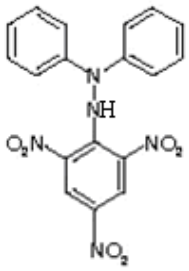
1.7.3.1 The DPPH Assay

The radical-scavenging activity of antioxidants may be influenced by the radical system and other testing conditions. Two or more radical systems are needed to better study a selected antioxidant for its radical scavenging properties. Free radical scavenging is one mechanism by which antioxidants inhibit lipid oxidation. Antioxidant capacity of polyphenols in foods, vegetable, fruits and plant extract is usually tested with 1,1-diphenyl-2-picrylhydrazyl hydrazyl (α,α-diphenyl-β-picrylhydrazyl). $DPPH^{\bullet}$ (Fig. 1.4) is a stable free radical in a methanolic solution, and has been used to estimate the radical-scavenging capacities of antioxidants and to evaluate the kinetics and thermodynamic properties of radical-antioxidant reactions (Yu *et al.*, 2002). Scavenging of $DPPH^{\bullet}$ by antioxidant is due to their hydrogen-donating ability (Singh and Bhat, 2003). The $DPPH^{\bullet}$ method is performed in a polar medium such as methanol at ambient temperature without any additional oxygen (Brand-Williams *et al.*, 1995). The radical is stable because the spare electron is delocalized over the whole molecule. This

delocalization is responsible for its deep violet color. Because of its odd electron, the radical is paramagnetic. However, it can accept an electron or hydrogen radical to become stable and diamagnetic (Figures 1.6 and 1.7). This yields the reduced form with the loss of the deep violet color, occasionally giving a residual pale yellow color due to picryl group (Molyneux, 2004).



DPPH



DPPH

Figure 1.6 Diphenylpicrylhydrazine (free radical). **Figure 1.7** Diphenylpicrylhydrazine (non-radical).

The decreasing absorbance of the stable radical is monitored at a characteristic wavelength after the single occupied orbital is filled up with an electron provided by the antioxidant (Krings and Berger, 2001). The maximum absorption of $DPPH^{\bullet}$ occurs at 515 – 520 nm, which disappears as the unpaired electron becomes stabilized, or rather upon reduction by an antiradical compound. This is a decolouration assay produced by the addition of the antioxidant to a $DPPH^{\bullet}$ solution in ethanol or methanol. Absorbance measurement is not affected by the color of the extracts in the reaction medium. $DPPH^{\bullet}$ will oxidize ascorbic acid, tocopherols (Vitamin E), glutathione, polyhydroxy aromatic compounds and aromatic amines (Blois, 1958). Besides studying the sample extracts, a standard/positive control such as ascorbic acid or α-tocopherol should be included. Representing the DPPH radical by $Z^{\bullet}$ (where $Z^{\bullet}$ is as a free radical) and the donor molecule by ascorbic acid (Vitamin C), as an antioxidant, the primary and second reactions are shown in Figure 1.8. Two molecules of DPPH are reduced by one molecule of ascorbic acid.

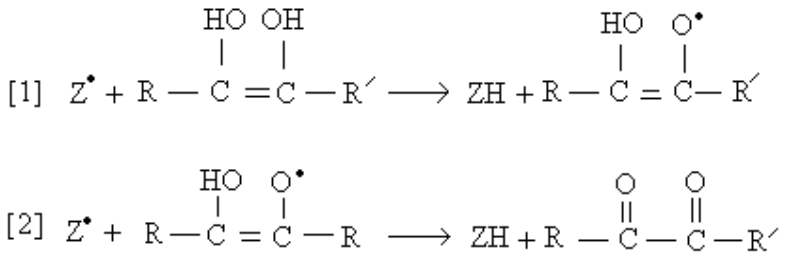


Figure 1.8 The neutralizing reactions free radical by ascorbic acid.

Sanchez-Moreno (Sanchez-Moreno *et al.*, 1999) proposed that the reaction be monitored until it has reached a plateau and the reaction kinetics plotted, followed by determination of percentage inhibition at steady state from these graphs. The values of percentage inhibition should then be transferred onto another graph showing the percentage inhibition as a function of antioxidant concentration. Finally, Molyneux (Molyneux, 2004), stated that usage of the EC_{50} value has a drawback because the higher the antioxidant activity, the lower the value of EC_{50} (substrate concentration to produce 50% reduction of the DPPH).

In an experiment shown that the DPPH scavenging activity of the unripe guava as measured by the AEAC value, is primarily due to its higher total phenol content (TPC) relative to ascorbic acid content (AAC) (Lim *et al.*, 2006). A decrease in AEAC during ripening suggests that the antioxidant activities of guava fruit declined during fruit ripening. They also compared antioxidant properties of guava with other tropical fruits and observed that both varieties of guava fruit contain relatively high quantity of antioxidants as shown by the high amount of TPC and AAC recorded. In the case of AAC, guava (*jambu batu*) contained as much as ten times the quantity of the antioxidant as that of other fruits such as banana; dragon fruit, star fruit and sugar apple have a comparable. On the whole, the results suggest that guava is a healthy fruit to consume from the antioxidant viewpoint, and is better than temperate fruits such as oranges and apples.

For antioxidant activities, this can be primary or secondary. Primary antioxidant properties are generally measured by the DPPH assay (expressed as AEAC and IC50) and FRAP. The DPPH assay measures the ability of the fruit extract to donate hydrogen to the DPPH radical resulting in bleaching of the DPPH solution. The greater the bleaching action, the higher the antioxidant activity (AEAC value), and this is reflected in a lower IC50 value.

Kondo (Kondo *et al.*, 2005) reported that DPPH-radical scavenging activity in the skin of guavas, mangoes, and papayas had lower IC50 values than those in the flesh throughout development. However, banana skin had a higher DPPH IC50 value compared to the flesh, and DPPH IC50 of the skin and flesh in rose apples (*Syzygium jambus* Alston) showed no significant difference except at 56 days after full bloom (harvest).

Kulkarni (Kulkarni and Aradhya, 2005) working on pomegranate arils showed a rapid decrease in antioxidant activity (by 13%) during 20 to 60 days of fruit development, which immediately replenished to its peak activity with 10.6% increase on the 80th day. The lowest antioxidant activity (61.6%) was recorded in 60 day-old fruits, probably due to a reduced concentration of total phenolics and ascorbic acid in the arils, (73.9% and 80.1%) respectively.

It has been reported that results of the DPPH assay showed that red chili spur pepper had the highest antioxidant capacity, followed by bird chili (red and green) holy basil (red and white), green chili spur pepper, garlic and pumpkin, respectively (Wangcharoen and Morasuk, 2007).

1.7.3.2 The ABTS Assay

The ABTS assay is also designed to measure the overall antioxidant capacity within a given sample. The assay relies on the ability of antioxidants in the sample to

inhibit the oxidation of ABTS (2,2 □-azino-di-[3-ethylbenzthiazoline sulphonate]). A method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, and carotenoids. The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) is generated by oxidation of ABTS with potassium per sulfate and is reduced in the presence of such hydrogen-donating antioxidants (Fig. 1.9). The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity (Re *et al.*, 1999).

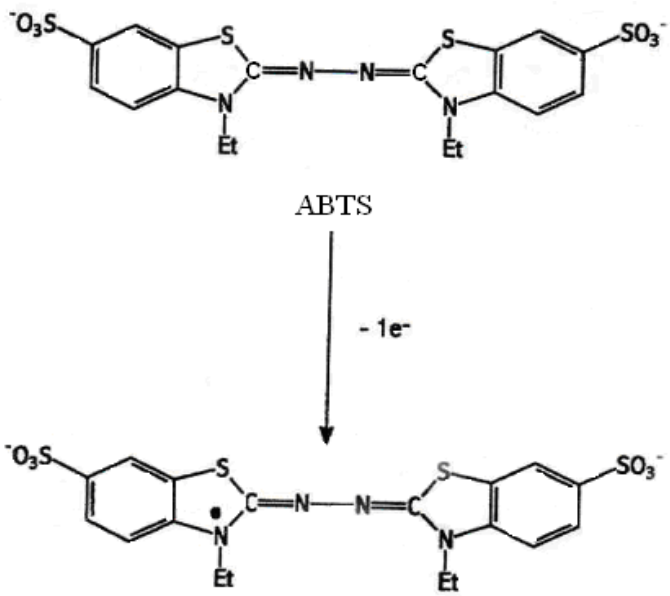


Figure 1.9 Oxidation of ABTS to $ABTS^{\bullet+}$ radical (Akerstrom *et al.*, 2007; Rojo *et al.*, 2009).

In an experiment carried out on guava fruits it was reported that correlations among methanol extract (AOAM) on the ABTS, DPPH, FRAP, and ORAC assays, were positively high and ranged between 0.68 and 0.97. The highest correlation was between ABTS and FRAP (0.97) and the lowest correlation was between DPPH and ORAC (0.68). ABTS and DPPH assays are based on the reduction of ABTS and DPPH

free radicals by the samples, but values from DPPH assay might be lower than those from the ABTS assay (Thaipong *et al.*, 2006).

It has been shown that some compounds which have ABTS scavenging activity may not show DPPH scavenging activity (Zhang *et al.*, 2008). Wangcharoen (Wangcharoen and Morasuk, 2007) reported that in the ABTS assay, the plants with the highest antioxidant capacity were bird chili (red and green) and red holy basil, followed by red chili spur pepper and white holy basil, green chili spur pepper, garlic, and pumpkin, respectively.

1.8 PAL Enzyme Activity

One of the most important enzymes in plant metabolism is phenylalanine ammonia-lyase, usually abbreviated as PAL. In plant metabolism one of the most central metabolic pathways is the Shikimic Acid Pathway which leads to the synthesis of the aromatic amino acids, namely, tyrosine, tryptophan and phenylalanine. In some plants it accounts for more than 50% of its metabolism because from it many important plant compounds, secondary metabolites are synthesized. PAL (EC number 4.3.1.24) catalyzes the following chemical reaction;

$$\text{L-phenylalanine} \rightarrow \text{trans-cinnamate} + NH_3$$

This enzyme has one substrate, L-phenylalanine, and two products, Trans cinnamate and ammonia (NH_3). PAL enzyme belongs to the family of lyases, specifically ammonia lyases, which cleave carbon-nitrogen bonds. The systematic name of this enzyme class is L-phenylalanine ammonia-lyase (trans-cinnamate-forming). Other names in common use include tyrase, phenylalanine deaminase, tyrosine ammonia-lyase, L-tyrosine ammonia-lyase, phenylalanine ammonium-lyase, PAL, and L-phenylalanine ammonia-lyase. This enzyme participates in 5 metabolic pathways:

tyrosine metabolism, phenylalanine metabolism, nitrogen metabolism, phenylpropanoid , and alkaloid biosynthesis (Fig. 1.10).

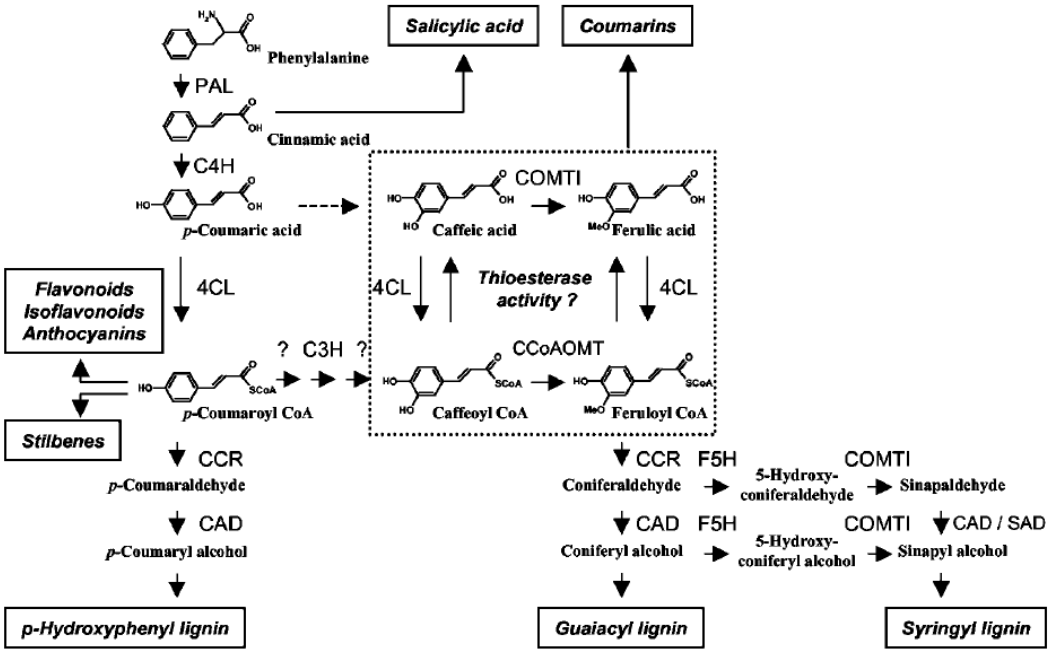


Figure 1.10 Outline of the phenylpropanoid biosynthetic pathway. *4CL*, 4-hydroxycinnamoyl-CoA ligase; *C3H*, *p*-coumarate 3-hydroxylase; *C4H*, cinnamate 4-hydroxylase; *CAD*, cinnamyl-alcohol dehydrogenase; *CCoAOMT*, caffeoyl-CoA *O*-methyltransferase; *CCR*, cinnamoyl-CoA reductase; *COMTI*, caffeic/5-hydroxyferulic acid *O*-methyltransferase I; *F5H*, ferulate 5-hydroxylase;*PAL*, phenylalanine ammonia-lyase; *SAD*, sinapyl-alcohol dehydrogenase.Taken from Hoffmann (Hoffmann *et al.*, 2003).

Phenolic compounds such as flavonols, anthocyanins and phenolic acids, including benzoic, cinnamic and coumarin represent the largest group of plant secondary metabolites that embrace a variety of structural classes, for example as precursors of lignin and they originate from trans-cinnamic acid, which is produced by the action of PAL, the key regulatory enzyme in the first stage of the phenylpropanoids pathway. It links the primary metabolism to the secondary metabolism by catalyzing the deamination of the primary metabolite L-phenylalanine to produce trans-cinnamic acid, thus, leading to the formation of a wide range of secondary metabolites with the phenylpropane structure, e.g. lignin, which impregnate xylem cell walls during differentiation and suberins which are integral constituents of the cell wall matrix in

endodermal and phellogen tissues, and a wide variety of natural products such as anthocyanins, absorbent of ultraviolet (UV), phytoalexins, phenolic compounds and flavonoids (Singleton and Esau, 1969; Lister *et al.*, 1996).

The development of the red pigmentation with maturity in some fruits is dependent on an increase anthocyanin during the maturing period (Wang *et al.*, 2000). Anthocyanin synthesis is a process that involves many steps from the primary precursor (phenylalanine) to the end products, glycosides of cyaniding (Wang *et al.*, 2000). As mentioned above PAL is the first enzyme to catalyse the elimination of NH_3 from L-phenylalanine to give trans-cinnamate. PAL activity has been reported to positively correlate with anthocyanin synthesis in grapes (Kataoka *et al.*, 1983), strawberries (Given *et al.*, 1988) and apples (Faragher and Chalmers, 1977; Arakawa *et al.*, 1986).

1.9 Objectives of Study

Currently there is little information in the literature about the postharvest physiological and biochemical characteristics on the *jambu air* fruit (*Syzygium samarangense)*, a fruit that has become increasingly important in this region. This study aims to investigate the following parameters;

1. Changes in physiological and biochemical characteristics that occur in the *Syzygium samarangense* fruit during ripening and postharvest storage.
2. To determine the antioxidant activity in the *Syzygium samarangense* fruit using an improved 2,20-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical decolourization assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay.
3. To determine and correlate phenylalanine ammonia lyase (PAL) enzyme activity in *jambu air* fruit during storage, with total phenolic, flavonoid content and colour development.